AVIAN DISEASES 51:674–680, 2007

Periodic Monitoring of Commercial Turkeys for Enteric Viruses Indicates Continuous Presence of Astrovirus and Rotavirus on the Farms

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Received 9 January 2007; Accepted and published ahead of print 20 February 2007

SUMMARY. A longitudinal survey to detect enteric viruses in intestinal contents collected from turkeys in eight commercial operations and one research facility was performed using molecular detection methods. Intestinal contents were collected from turkeys prior to placement, with each flock resampled at 2, 4, 6, 8, 10, and 12 wk of age. The samples were screened for astrovirus, rotavirus, reovirus, and turkey coronavirus (TCoV) by a reverse transcriptase and polymerase chain reaction (RT-PCR), and for groups 1 and 2 adenovirus by PCR. Rotavirus was the only virus detected prior to placement (7 of 16 samples examined). All of the commercial flocks were positive for rotavirus and astrovirus from 2 until 6 wk of age, and most were intermittently positive until 12 wk of age, when the birds were processed. Of the 96 samples collected from birds on the farms, 89.5% were positive for astrovirus, and 67.7% were positive for rotavirus. All flocks were negative for TCoV, reovirus, and group 1 adenovirus at all time points, and positive for group 2 adenovirus (hemorrhagic enteritis virus) at 6 wk of age. All the flocks monitored were considered healthy or normal by field personnel. Turkeys placed on research facilities that had been empty for months and thoroughly cleaned had higher body weights and lower feed conversion rates at 5 wk of age when compared to turkeys placed on commercial farms. Intestinal samples collected at 1, 2, and 3 wk of age from these turkeys were free of enteric viruses. This report demonstrates that astroviruses and rotaviruses may be present within a turkey flock through the life of the flock. Comparison of infected birds with one group of turkeys that were negative for enteric viruses by the methods used here suggests that astrovirus and/or rotavirus may affect production. The full impact on flock performance needs to be further determined.

RESUMEN. El análisis periódico de pavos comerciales para detectar virus entéricos, indica la presencia continua de Astrovirus y Rotavirus en las granjas.

Utilizando métodos de detección molecular, se realizó un estudio longitudinal para detectar virus entéricos en los contenidos intestinales de pavos provenientes de ocho empresas comerciales y de una instalación de investigación. Se tomaron muestras de contenidos intestinales de pavos antes de ser llevados al sitio de crianza, cada parvada fue muestreada nuevamente a las 2, 4, 6, 8, 10 y 12 semanas de edad. Las muestras fueron evaluadas mediante la prueba de reacción en cadena por la polimerasa-transcriptasa reversa para detectar la presencia de astrovirus, rotavirus, reovirus y coronavirus de pavo y mediante la prueba de reacción en cadena por la polimerasa para detectar adenovirus de los grupos I y II. El rotavirus fue el único virus detectado antes de llevar las aves a los sitios de crianza (7 de 16 muestras analizadas). Todas las aves comerciales resultaron positivas para rotavirus y astrovirus desde la segunda hasta la sexta semana de edad, mientras la mayoría resultó positiva de manera intermitente hasta la semana 12, cuando se procesaron las aves. De las 96 muestras tomadas de las aves en las granjas, 89.5% resultaron positivas para astrovirus y 67.7% eran positivas para rotavirus. En todos los muestreos, todas las parvadas resultaron negativas para coronavirus de pavo, reovirus, y adenovirus grupo 1 y positivas para adenovirus grupo II (virus de enteritis hemorrágica) a las seis semanas de edad. Todas las aves examinadas fueron consideradas como saludables o normales por el personal de campo. Los pavos colocados en las instalaciones de investigación que habían estado vacías por meses y habían sido desinfectadas exhaustivamente, mostraron mayor peso corporal y menor conversión alimenticia a las 5 semanas de edad, que los pavos colocados en las granjas comerciales. Las muestras intestinales tomadas de estas aves a la primera, segunda y tercera de edad resultaron libres de virus entéricos. El presente reporte demuestra que los astrovirus y rotavirus pueden estar presentes en una parvada de pavos a lo largo de la vida de la parvada. La comparación de las aves infectadas con un grupo de pavos que eran negativos para virus entéricos (mediante los métodos utilizados aquí), sugiere que los astrovirus y rotavirus pueden afectar la producción. El impacto global en el rendimiento requiere de determinaciones adicionales.

Key words: avian astrovirus, avian nephritis virus, avian rotavirus, turkey astrovirus, adenovirus, phylogenetic analysis

Abbreviations: aa = amino acid; ANV = avian nephritis virus; ARV = avian reovirus; EM = electron microscopy; HE = hemorrhagic enteritis; IFA = immunofluorescent assays; NCSU = North Carolina State University; nt = nucleotide; PBS = phosphate buffered saline; PEC = poult enteritis complex; PEMS = poult enteritis mortality syndrome; RT-PCR = reverse transcriptase and polymerase chain reaction; RVLV = rotavirus-like virus; SEPRL = Southeast Poultry Research Laboratory; SPF = specific-pathogen-free; TAstV = turkey astrovirus; TCoV = turkey coronavirus

Poult enteritis complex (PEC) is an economically important disease of young turkeys characterized by stunting, increased feed consumption, and increased time to market (1,2). In the more severe forms, runting, immune dysfunction, and mortality occur (1). One of its manifestations, poult enteritis mortality syndrome (PEMS), has caused devastating economic losses to the turkey industry sporadically since 1991 (1). Independent of PEMS, poult enteritis and stunting continue to be a common problem in young commercial turkeys throughout the United States and cause great economic loss, mostly from the failure of turkeys to reach their full genetic growth potential. PEC is believed to be a polymicrobial disease, and several enteric viruses have been associated with the condition. The most important viruses associated with poult enteritis are turkey coronavirus (TCoV) (1,2,6,14), turkey astrovirus (TAstV) (3,8,15,18), avian reovirus (ARV) (2,4), rotaviruses (11,28), and

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adenoviruses (5,22,24). The role most of these enteric viruses play in poult enteritis and stunting remains unclear. In previously published studies on the prevalence and distribution of enteric viruses in commercial turkeys the detection methods used had limited sensitivity and did not detect all enteric viruses (16,17,19,27, 28,29). Current information on enteric viruses in commercial turkeys is essential for the control of enteric disease.

Detection of avian viruses by molecular techniques has become routine in most diagnostic laboratories. Traditionally diagnosis of viral enteric infections in turkeys has been made by electron microscopy (EM), immunofluorescent assays (IFA), and genome electropherotyping to detect and identify the viruses and by ELISA to detect antibodies. There are many advantages to using RT-PCR for detecting enteric viruses over traditional methods, including greater sensitivity and specificity, detection of multiple viruses in one sample, no need for virus propagation, the ability to test a large number of samples quickly, and reduced cost of the assay. Currently molecular-based diagnostic tests are available for TCoV, TAstV-2, ARV, and adenoviruses (5,9,20,23,24,26). In this study we used previously published molecular tests to detect TCoV, astrovirus, reovirus, and adenovirus in intestinal samples, and we developed a RT-PCR test for detection of avian rotaviruses.

The objective of this study was to observe the age-associated patterns of infection for several enteric viruses including astroviruses, rotaviruses, reoviruses, turkey coronavirus, and group 1 and 2 adenoviruses in commercial turkeys and turkeys reared under optimal biosecurity conditions. To accomplish this, eight commercial light hen turkey flocks and one flock reared on a research farm were monitored prior to placement and at biweekly intervals for enteric viruses throughout grow-out with molecular diagnostic tests. Phylogenetic analysis of the viruses detected in this study was also performed.

MATERIALS AND METHODS

Sample origin and collection. Intestinal contents were collected from light turkey hens from eight different flocks in North Carolina during the year 2005 (May–June). Flocks belong to separate farms that were managed by the same operation but employed separate personnel for each farm. Poults were delivered to the brooding facility within 30 hr following hatch. Samples consisted of pooled intestines from three birds from the front of a house and three birds from the back of the house from each of the eight farms and were collected at 2, 4, 6, 8, 10, and 12 wk of age, for a total of 96 samples. Sixteen additional samples were also taken at the hatchery from poults before placement in the respective farms. Samples were preserved at −70 C until shipment on wet ice (by overnight courier) to Southeast Poultry Research Laboratory (SEPRL, USDA, Athens, GA).

Intestinal contents were also collected from turkey poults placed on a research farm at North Carolina State University (NCSU). These poults came from the same hatchery as the poults placed in the commercial farms. The facilities at NCSU had been thoroughly cleaned and disinfected and had remained free of birds for 5 mo prior to placing the birds. Samples were collected at placement and at 1, 2, and 3 wk of age. Performance data (weight at 5 wk of age and feed conversion) were obtained from the producer on commercial flocks and from NCSU.

Viruses. Astrovirus isolates collected from 2003 to 2005 in the United States from the SEPRL repository were used as positive controls for the RT-PCR tests for astroviruses (12). Rotaviruses isolated at SEPRL and identity confirmed by sequencing were used as controls for the RT-PCR tests for rotavirus. The turkey coronavirus (TCoV) reference isolate VA/SEP-C26/03 from the SEPRL repository was used for the TCoV tests (23). Two reference isolates were used for the reovirus RT-PCR tests: S1133 and NC/98 (23). Group 1 and group 2 avian adenovirus isolates also from the SEPRL repository were used for the adenovirus tests, identities also confirmed by sequencing. For all molecular tests performed, negative controls consisted of intestinal contents collected from specific-pathogen-free (SPF) turkeys raised at SEPRL.

RNA extraction. Two hundred microliters of intestinal contents were diluted in 1.2 ml of phosphate buffered saline (PBS), homogenized with sterile glass beads in a FastPrep homogenizer (Savant, Inc.), and centrifuged for 10 min at 800 × *g*. Total RNA was extracted directly from 250 µl of the supernatant using Trizol™ total RNA isolation reagent (Invitrogen Inc., Carlsbad, CA) according to the manufacturer's instructions.

RT-PCR for astroviruses. Segments of the polymerase and capsid genes of turkey astroviruses were amplified by standard RT-PCR using the Qiagen one-step RT-PCR kit (Qiagen Inc., Valencia, CA) and primers directed to these genes. Two set of primers were used for detecting astroviruses. The first set of primers were designed by Tang *et al.* (26) and detect TAstV-1, TAstV-2, and avian nephritis virus (ANV) (12) and amplify a 601-nucleotide (nt) region of the polymerase gene; they are designated TAP-L1 and TAPG-R1. The second set of primers used, MKCap8 and MKCap19, amplify a 849-base-pair region from within the viral capsid gene of TAstV-2 (9). Amplifications were performed in a MJ Research DNA thermocycler (Waltham, MA), and the incubation steps consisted of 50 C for 30 min, 94 C for 2 min, and then 35 cycles of annealing at 50 C for 30 sec, extension at 72 C for 60 sec, and denaturation at 94 C for 30 sec. The RT-PCR conditions consisted of 1X Qiagen OneStep RT-PCR kit reaction buffer, 320 µM of each dNTP, 0.6 µM of each primer, and 1 µl of Qiagen RT-PCR enzyme blend and 2.5 µl of extracted RNA, for a total volume of 25 µl.

The PCR products were separated on an agarose gel by electrophoresis, and amplicons of the appropriate size were subsequently excised from the gel and extracted with the QIAquick gel extraction kit (Qiagen). Purified PCR products were subjected to direct sequencing. Sequencing was performed in both directions with the same primers used in the RT-PCR reactions, using the BigDye terminator kit (Applied Biosystems, Foster City, CA) run on an ABI 3730 sequencing machine (Applied Biosystems).

RT-PCR for rotaviruses. Alignment of NSP4 gene sequences among different avian rotaviruses was conducted to identify the variable and conserved regions. A set of primers was designed to amplify a 630-bp product: NSP4 F30 (5′-GTGCGGAAAGATGGAGAAC-3′) and NSP4 R660 (5′-GTTGGGGTACCAGGGATTAA-3′). RT-PCR was done following the same protocol used for amplifying astroviruses.

Multiplex RRT-PCR for ARV's and RRT-PCR for TCoV. Primer and probes used are referenced in Spackman *et al.* (23). Primers for ARV target the S1 gene segment of chicken-origin reoviruses and the S3 gene segment of turkey-origin reoviruses. Primers for TCoV target the matrix gene. RRT-PCR was run on a Cepheid Smart Cycler (Cepheid Inc., Sunnyvale, CA) in a 25-µl volume with the Qiagen OneStep RT-PCR kit (Qiagen). The reverse transcription step was the same for all tests: 50 C for 30 min and 95 C for 15 min. The TCoV reaction conditions were as follows: 1X Qiagen OneStep RT-PCR kit reaction buffer, 320 mM dNTP mix, 3.75 mM magnesium chloride, 20 pmol of each primer, 0.2 µM TCoV probe, and 1 µl of Qiagen RT-PCR enzyme blend. The ARV-S1-S3 reaction conditions were as follows: 1X Qiagen OneStep RT-PCR kit reaction buffer, 320 mM dNTP mix, 3.75 mM magnesium chloride, 10 pmol of each primer, 0.1 µM ARV S1 probe, 0.2 µM ARV S3 probe, and 1 µl of Qiagen OneStep RT-PCR enzyme blend. Fluorescence data were acquired at the end of each annealing step using the Smart Cycler (Cepheid).

PCR for avian adenoviruses. Primer sets previously described were used for the detection by PCR of group 1 adenoviruses (fowl adenovirus) and group 2 adenoviruses (hemorrhagic enteritis virus) (5,24). DNA extraction from samples was performed using saturated phenol:chloroform. Briefly, 250 µl of the 1:10 intestinal homogenate was mixed with 250 µl of saturated phenol: chloroform (1:1); the resultant emulsion was vortexed for 10 sec and centrifuged 14,000 × *g* for 3 min. The aqueous phase was removed to a fresh tube. An equal volume of chloroform was added, vortexed, and centrifuged 14,000 × *g* for 3 min. The aqueous phase was removed to a fresh tube, and 2.5–3

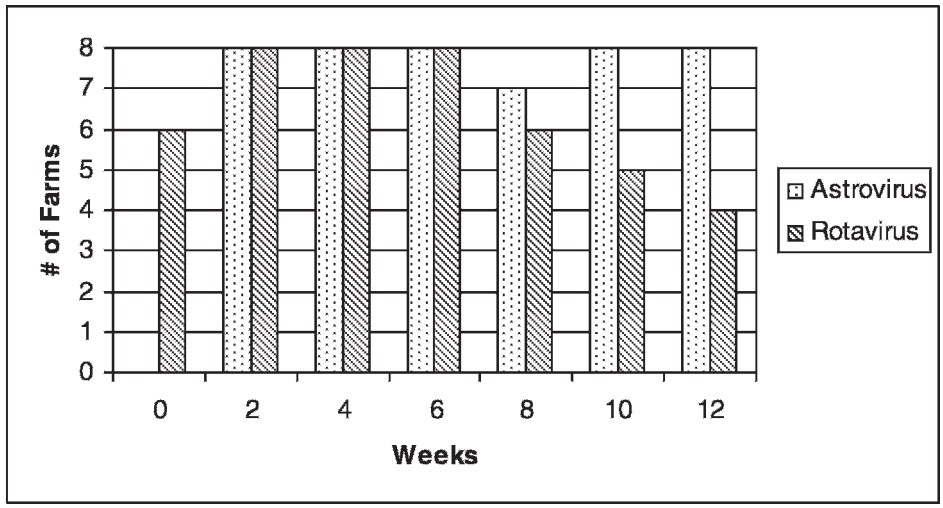

Fig. 1. Number of farms in which astroviruses and rotaviruses were detected. Samples were taken from poults before placement (week 0) and at 2-wk intervals (2–12 wk).

volumes of 95% ethanol/0.12 M sodium acetate was added, inverted to mix, incubated on ice a minimum of 10 min, and centrifuged at 14,000 $\times$ *g* for 15 min at 4 C. Supernatant was decanted and 80% ethanol added, corresponding to two volumes of the original sample, and then incubated at room temperature for 10 min. Samples were then centrifuged at 14,000 $\times$ *g* for 5 min. Supernatant was decanted, and the pellet was dried in a speed-vac for 10 min at 37 C. DNA was hydrated with 100 µl nuclease-free water and stored at −80 C until further analyzed.

DNA amplifications were carried out in a total volume of 25 µl containing 50 ng total DNA as determined by UV spectroscopy, and 125 pmol of each primer. PCR reactions were run using the Qiagen Platinum PCR SuperMix High Fidelity Kit (Qiagen). A total of 34 cycles were carried out in a MJ Research thermocycler (Waltham), beginning with an initial denaturation step of 2 min at 94 C. The temperature profile of each cycle included denaturation of 1 min at 94 C and annealing at 55 C for 45 sec to 1 min. Extensions steps in all PCRs were 68 C for 1 min per kb, minimum 1 min. The PCRs were finished with a final extension step of 10 min at 68 C. An aliquot of each PCR reaction was separated by electrophoresis in an agarose gel and visualized by UV transillumination.

Sequence analysis of amplicons. A 423-nt region of the ORF 1b (from 4002 to 4425, numbering based on the TAstV-1 genome, NC002470) was used to evaluate the phylogenetic relationships among the detected avian astroviruses. Segments of the polymerase gene and protein from published avian astrovirus sequences in GenBank were included in the phylogenetic analysis: TAstV-1 complete genome (NC002470), TAstV-2 complete genome (NC005790) (NC/96), TAstV-2 polymerase partial sequence (AY320042) (TEVL-NC88), ANV-1 complete genome (NC 003790), TAstV-2 polymerase partial sequences (DQ066576 to DQ066598) (13), and avian astroviruses polymerase partial sequences (DQ324802 through DQ324850) (12).

The capsid genes (nucleotide position 6208 to 7035, based on NC/96 numbering NC005790) of samples positive for TAstV-2 by RT-PCR with the MKCap and MKPol primers were also sequenced and analyzed. Segments of the capsid gene and protein from published avian astrovirus sequences from GenBank were included in the phylogenetic analysis: TAstV-1 complete genome (NC002470), TAstV-2 complete genome (NC005790 (NC/96), and TAstV-2 capsid partial sequences (DQ066553 to DQ066575) (13).

A 558-nt-long region of the NSP4 gene of rotaviruses (from 18 to 575, numbering based on Ty-3 NSP4 gene, AB065286) was used to evaluate the phylogenetic relationships among the detected avian rotaviruses. Segments of the NSP4 gene and protein from published avian rotavirus sequences from GenBank were included in the phylogenetic analysis: turkey rotaviruses strains Ty-1 (AB065285) and Ty-3 (AB065286), chicken rotavirus strain Ch-1(AB065287), avian rotavirus strain AvRV-1 (AY062937), and pigeon rotavirus strain PO-13 (AB009628).

The sequence information was compiled with the Seqman progam (Lasergene 7.0, DNASTAR, Madison, WI), and the nucleotide and amino acid sequences were aligned initially with the Megalign application of the same software package using the Clustal W alignment algorithm (Slow/Accurate, Gonnet). Pairwise sequence alignments were also performed in the Megalign program to determine nucleotide and amino acid sequence identity. Phylogenetic analysis was performed with PAUP* 4.0b10 (Sinauer Associates, Inc., Sunderland, MA) using the maximum parsimony tree building method by a heuristic search with 500 bootstrap replicates (25).

GenBank accession numbers. The rotavirus nucleotide sequences presented in this article have been submitted to GenBank under accession numbers ES204132 to EF204143.

RESULTS

Virus detection. The detection of astroviruses and rotaviruses by age in commercial turkeys is shown in Fig. 1. Rotavirus was the only virus detected prior to placement (7 of 16 samples examined). All of the commercial flocks were positive for rotavirus and astrovirus from 2 to 6 wk of age, and all were intermittently positive until 12 wk of age when the birds went to slaughter. Of the 96 samples examined throughout the monitoring period, 89.5% were positive for astrovirus, and 67.7% were positive for rotavirus. Five of eight farms were positive for group 2 adenovirus (hemorrhagic enteritis virus) at 6 wk of age. All flocks were negative for TCoV, ARVs, and group 1 adenoviruses at all time points.

Intestinal samples collected from turkeys poults placed on the research facilities were free of the enteric viruses mentioned when examined at 1, 2, and 3 wk of age.

Phylogenetic analysis. Phylogenetic analysis was performed on most of the astrovirus-positive samples and included nt and amino acid (aa) avian astroviruses ORF1b and ORF2 sequences available in GenBank. The phylogenetic assortment of the nt sequences was similar to that of the aa sequences indicating coding changes. The astroviruses detected assorted into three groups based on the polymerase gene (ORF1b): TAstV-1-like, TAstV-2-like, and

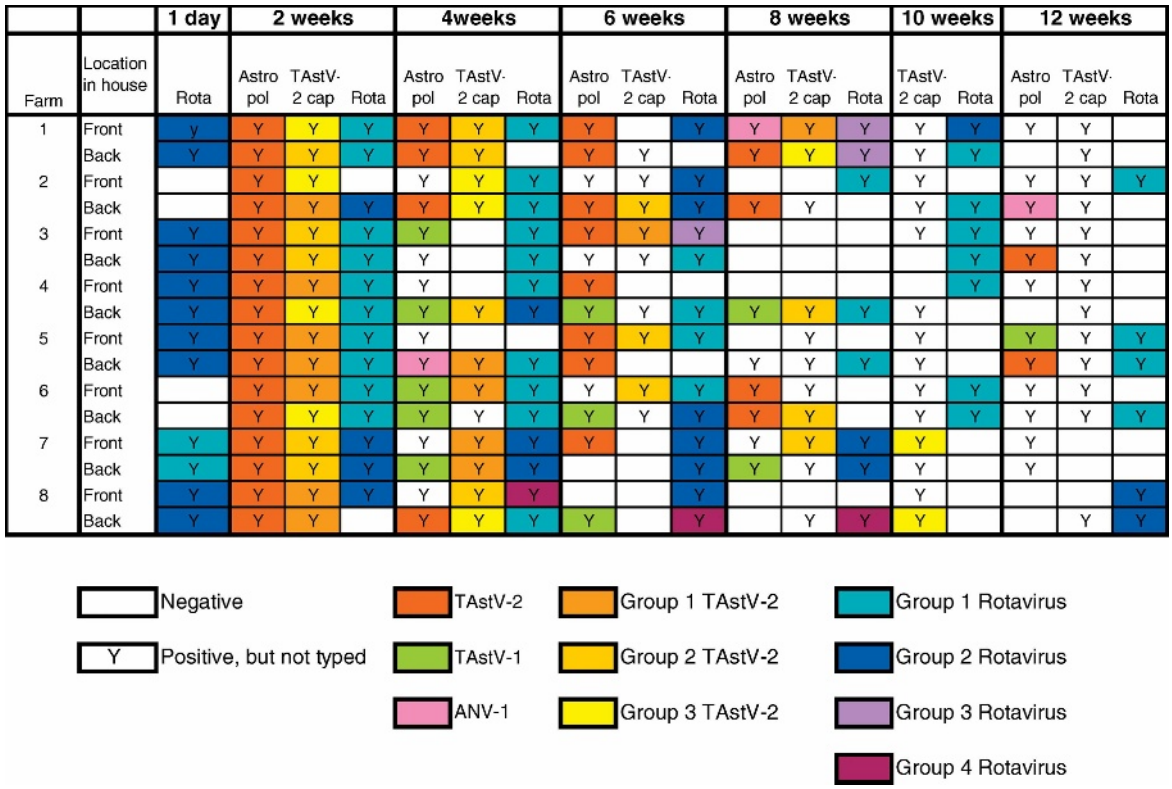

Fig. 2. Astroviruses and rotaviruses detected by RT-PCR in commercial turkey flocks before placement and every 2 wk until 12 wk of age. Colored boxes represent genetic types identified by sequencing.

ANV-1-like (Fig. 2) (12). TAstV-2 was also detected by the use of a set of primers that amplified the capsid gene (ORF2). TAstV-2 was the virus most commonly identified, with 78 of the 112 samples analyzed positive by RT-PCR, and of those, 48 confirmed by sequencing. TAstV-1 and ANV were less frequently identified (11 and 3 of 112, respectively). TAstV-2 was detected in all farms at 2 wk of age and in six or more farms at all other time points. TAstV-1 was detected on five different farms between 4 and 12 wk of age. ANV was detected on three different farms at 4, 8, and 12 wk of age. Only 26 samples of the total 112 analyzed, including samples taken from poults before placement, were negative for astrovirus. Based on the capsid gene, and compared to previous phylogenetic analysis of TAstV-2 (13), the TAstV-2s assorted into three major groups (Fig. 2); however, extensive genetic variation was observed among isolates from each group (data not shown). The phylogenetic groups of astroviruses detected in this study were consistent with those previously reported (13). The groups were distributed among the flocks, and multiple groups could be detected in the same flock at different time points; at least two groups of TAstV-2 were identified in each flock, and on two farms three groups were identified throughout the study (Fig. 2).

Rotaviruses were detected using of a set of primers that amplify the well-conserved NSP4 gene. Rotaviruses were detected in 75 of 112 samples examined, including samples taken from poults before placement. The number of infected flocks declined after the sixth week of age until only half of the farms were positive for rotavirus at 12 wk of age. Most of the samples examined had mixed infections of astrovirus and rotavirus (Fig. 2).

Phylogenetic analysis of the rotaviruses detected included all available nt and aa avian rotavirus NSP4 sequences from GenBank. The rotaviruses detected assorted into four groups (Fig. 3). Similar to astrovirus, multiple genetic groups could be detected in a single flock throughout the study. Among all four groups there was an nt identity of between 96.1% and 97.5% and an aa identity of between 97.5% and 99.5%. The turkey rotaviruses detected in this study had between 93.5% and 96.2% aa identity with the previously reported turkey rotavirus Ty-3, 86% and 86.6% with turkey rotavirus Ty-1, and between 76.3% and 76.9% with Ch-1.

Flock performance data. All commercial flocks were described as healthy and normally performing flocks by field personnel. However, compared to the turkeys raised on commercial farms, birds raised on the research facilities were on average 1.25–1.75 lb heavier at 5 wk of age and had a better feed conversion rate.

DISCUSSION

The results obtained in this study are similar to previously published reports on the prevalence of enteric viruses in commercial turkeys in that astroviruses and rotaviruses are the most frequently identified viruses in turkey flocks (16,17,28,29). However, these earlier studies were conducted using electron microscopy and electropherotyping as diagnostic methods, which are not as sensitive as the molecular techniques used today for viral diagnosis. Thus, the prevalence of astroviruses and rotaviruses in healthy flocks, as demonstrated in the present study, is much higher than previously reported (16,17,19,29). Additionally, this study follows commercial birds through grow-out to characterize the age association of some enteric viruses. The presence of rotavirus in samples collected from poults before placement could indicate possible vertical transmission of this virus. Previous studies have suggested this possibility (16,28). Fecal residues on hatchery eggs containing rotavirus could also act as

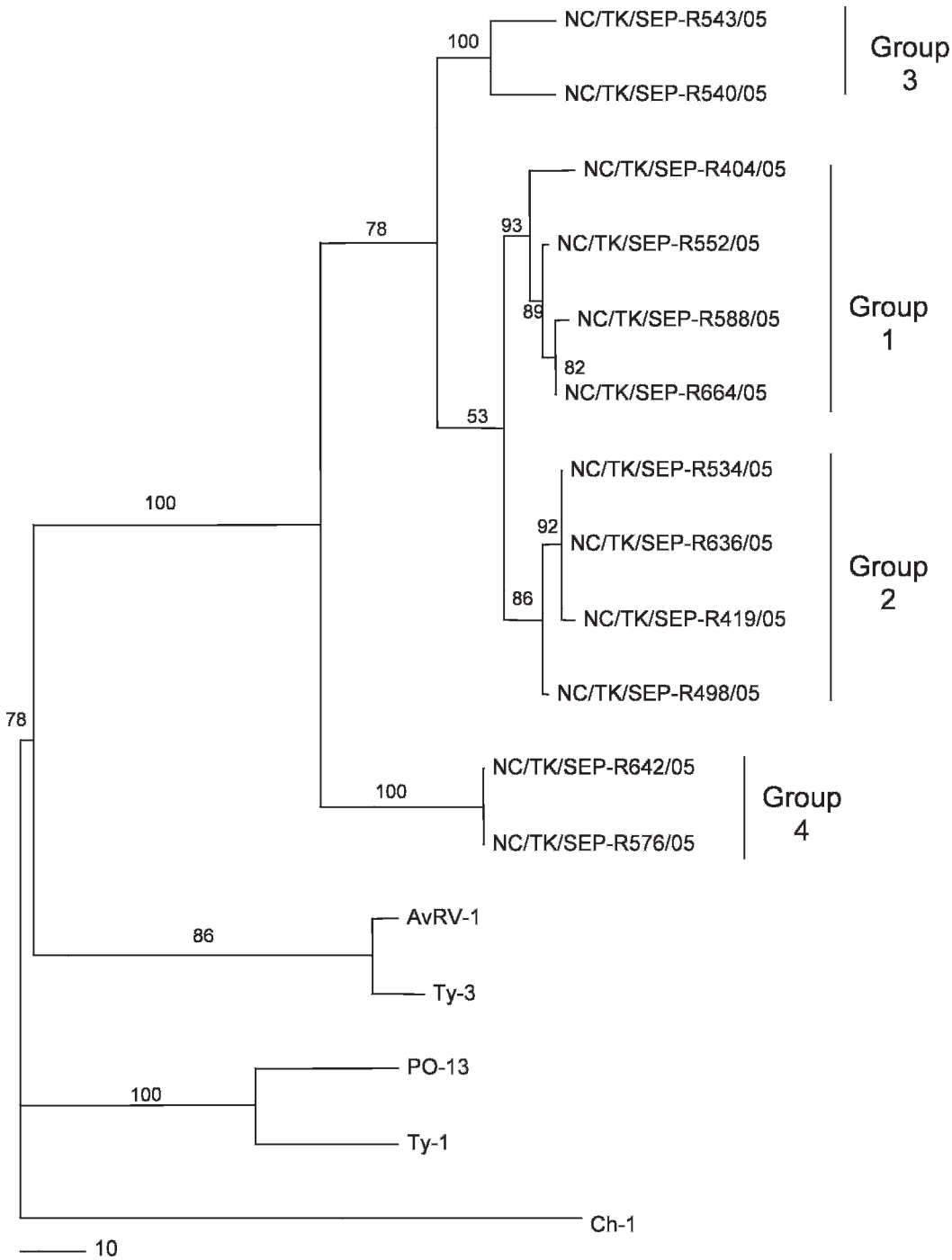

Fig. 3. Unrooted phylogenetic tree of ARV NSP4 nt sequence from selected representative isolates and reference isolates. The tree was constructed with PAUP* 4.0b10 (Sinauer Associates) using maximum parsimony, heuristic search, and 500 bootstrap replicates (bootstrap values are shown on tree). Isolate nomenclature is as follows: state of origin/species of origin/case number/year of isolation.

potential source of infection for newly hatched poults (16). Additional studies are required to determine whether rotaviruses are transmitted to poults via the eggs (in, or on the surface).

Molecular characterization of the viruses was performed by partial sequence analysis of both the polymerase and capsid genes of the astroviruses and the NSP4 gene of the rotaviruses. Three types of avian astroviruses were identified: TAstV-1, TAstV-2, and ANV. The most commonly identified astrovirus was TAstV-2, which had a high level of genetic variation, particularly in the capsid gene. Interestingly, more than one genotype was detected at the same time on the same farm. This indicates that more than one genotype of TAstV-2's are circulating in commercial turkey farms as previously suspected (13). TAstV-1, which has not been reported in turkeys since it was first isolated in 1985 (15,18), and ANV, which has previously been isolated only from chickens, were also identified. The significance of these two viruses in turkeys is still not known, and further characterization is needed.

Although the rotavirus NSP4 gene is somewhat conserved, with 96.1%–97.5% nt identity among the studied isolates, phylogenetic analysis of this gene showed that several variants are circulating in

farms. Similar to astrovirus, more than one genotype could be identified on the same farm at the same or different time points. The genetically most similar reference strain was turkey rotavirus Ty-3. It is likely that most of our isolates are group D avian rotaviruses, also referred to as rotavirus-like viruses (RVLV), since these have been the most commonly found rotaviruses in turkeys, as reported in previous studies (10,16,17,27). However, it is not known if avian rotaviruses of different serotype groups are present, since NSP4 is not a group-specific gene.

The identification of numerous astrovirus and rotavirus types and genetic variants within a single flock over time is probably not due to the virus changing over time on a farm or within a flock, but is likely due to the fact that only one of the numerous genetic variants present in a flock at any given time will be detected. It may be argued that the type identified is a predominant type, but detection of one variant instead of another at any given time is probably by chance. Circulation of numerous genotypes of a virus type appears to be a biological characteristic of some RNA viruses and is not well understood.

In a similar study published in 1986 by Reynolds *et al.* (17), intestinal samples from 91 turkey flocks between 1 day and 5 wk of age were examined for enteric viruses using EM and electropherotyping. Astroviruses with RVLV were the most frequently identified combination in both diseased and healthy flocks; however, 48% of the samples from normal/healthy flocks were negative. The combination of astrovirus and group D rotavirus infections in turkeys has also been reported by other investigators (19,29). In 2000, Yu *et al.* (29) examined intestinal samples from turkey poults affected with PEMS by EM and electropherotyping. Of the samples examined, 72% were positive for rotaviruses and 44% for small round viruses; but also 22% of the samples were positive for TCoV and 14% for reoviruses. In the present study, TCoV and reoviruses were not detected. TCoV has been strongly associated with enteric disease; however, this virus is relatively easy to diagnose and eradicate, so it is rarely found in healthy turkeys. Reoviruses also have been implicated in causing poult enteritis (4), and some reovirus isolates appear to be genetically distinct from previously characterized chicken and duck origin ARVs (7,21). Very little pathogenesis data are available for these viruses.

Hemorrhagic enteritis (HE) is an economically important disease caused by a group 2 adenovirus and occurs in turkeys 4 wk of age and older (22). Clinical outbreaks are characterized by intestinal hemorrhages accompanied with immunosuppression. The commercial flocks examined in this study were vaccinated at 4.5 wk of age with an attenuated live vaccine and were positive by PCR at 6 wk of age. Most likely the vaccine strain was detected.

Turkey poults from the same source and raised under similar conditions to turkeys raised commercially, but housed in cleaned and disinfected facilities, grew faster, and had better feed conversion rates. These birds were free of the viruses targeted in this study until at least 3 wk of age; this suggests that early exposure to astrovirus and/or rotavirus may impact production. However, improved performance in this flock could also be due to the absence of other viral, bacterial, or protozoal pathogens not examined in this study.

In conclusion, this study presents the age-associated patterns of infection with numerous enteric viruses in commercial turkey operations in North Carolina using sensitive and specific molecular detection methods. The presence of rotaviruses and astroviruses in commercial turkeys was common through the life of a flock. Furthermore, multiple genotypes and genetic variants of astroviruses and rotaviruses co-circulate on a given farm. Although further investigations to determine the effect of these viruses on turkey health and production are needed, it appears that early exposure to these viruses may have a negative impact on commercial turkey production.

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ACKNOWLEDGMENTS

We acknowledge Diane Smith, Scott Lee, and the SAA sequencing facility for technical assistance with this work. The work was supported by USDA/ARS CRIS project 6612-32000-020. Mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.